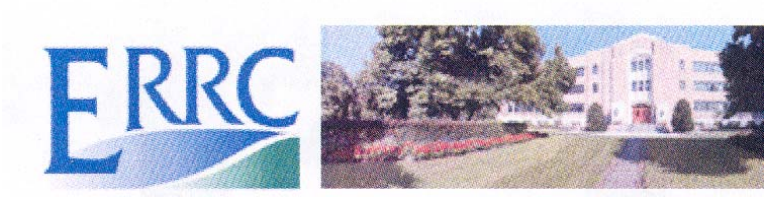

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Title: Sources of Bacterial Contamination on Apples, and Novel Approaches for Reduction of the Bacterial Contaminants

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SOURCES OF BACTERIAL CONTAMINATION ON APPLES, AND NOVEL APPROACHES FOR REDUCTION OF THE BACTERIAL CONTAMINANTS

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ABSTRACT

Outbreaks of foodborne illness, associated with the presence of *Escherichia coli* O157:H7 in unpasteurized apple cider, have led to increased interest in potential reservoirs of this pathogen in the orchard. Fruit samples (n = 63) (8 apple and 2 pear varieties), soil, water and fecal samples (collected from fourteen U.S. orchards), were analyzed for generic *E. coli*, total coliforms, total aerobic microflora, and yeasts and molds. Samples positive for generic *E. coli* and/or coliform were enriched and tested for *E. coli* O157:H7. Fruit was also tested for internalization of microflora by aseptically removing the core, stem and calyx areas and assessed the individual sections for the categories of microflora listed above. Generic *E. coli* was detected in 40, 43 and 6% of soil, water, and fruit samples tested, respectively. However, no *E. coli* O157:H7 was found. Coliforms were found in 74% of fruit samples, and were internalized in the cores of 40% of fruit tested. Yeasts and molds were internalized in 96.7% of samples and aerobic bacteria in 89.6%. Generic *E. coli* was not found to be internalized. Total aerobic counts and total coliforms were higher in dropped and damaged apples ($P < 0.05$) and in orchards associated with fecal contamination or proximity to pastures ($P < 0.05$). Findings suggest that dropped and/or damaged fruit should not be included in fruit designated for the production of unpasteurized juice, and that orchards should be located away from potential sources of contamination, such as pastures.

One of the novel fruit washing technologies under investigation in our laboratory is vacuum infiltration of sanitizing agents into produce. Preliminary results with vacuum infiltration of hydrogen peroxide into artificially inoculated Golden Delicious apples were encouraging.

INTRODUCTION

Since 1991 there have been several outbreaks of foodborne disease associated with *E. coli* O157:H7 in unpasteurized apple cider. These outbreaks were particularly significant, as they occurred in a highly acidic food product, previously thought to be safe because of its low Ph. However, studies have shown that *E. coli* O157:H7 can survive in unpasteurized apple cider produced in the traditional manner. The fact that this is a ready-to-eat product, receiving no further processing before consumption, is a matter of concern. As a consequence of these outbreaks the Food and Drug Administration (FDA), has mandated that juice products be treated with a process designed to yield a 5 $\log_{10}$ reduction in the most resistant organisms of public concern. Products not treated in this way must carry a warning label informing consumers of the potential risk of foodborne disease associated with the product.

As a result of the FDA ruling, there has been considerable research effort expended in the development of intervention methods by which to achieve the target 5 $\log_{10}$ reduction during the processing of juice products. A recent survey of small scale apple cider producers, (those

producing less than 5000 gallons of cider annually), indicated that 80% of producers would be interested in using alternative technologies to help assure the safety of their products. Potential intervention methods include pasteurization, both UV and thermal, irradiation, use of anti-microbial treatments such as hydrogen peroxide, high pressure inactivation and modified storage procedures.

Although, pasteurization is the most reliable method used to reduce pathogen numbers by 5 $\log_{10}$ in juice products, only 22% of small-scale apple cider producers pasteurized their product. The capital expenditure required for the purchase of the necessary equipment, in addition to adverse changes to sensory characteristics of the product, are among the reasons cited for the low pasteurization rate. Identification of alternative, less expensive intervention methods would be of great benefit to such processors.

In this study, potential reservoirs for *E. coli* O157:H7 in the orchard environment, and efficacy of vacuum infiltration of sanitizing agents in reducing *E. coli* populations on artificially inoculated apples, were investigated. The microflora profile on fruit harvested from a given orchard will be affected by orchard management practices. Constituents of the orchard environment, including fecal matter, soil, irrigation and surface water, and windblown dust are potential contamination sources for fruit. However, mechanisms of contamination are speculative, and further investigation is required before appropriate interventions can be introduced to reduce the risk of contamination. A survey of fruit, orchard environments and orchard management practices was performed in order to identify any associations between management practices and the prevalence and profile of microflora on fruit and in the orchard environment. Bacterial attachment, biofilm formation, inaccessibility in stem and calyx areas, growth in punctures, and internalization limited efficacy of wash treatments of apples using sanitizing agents. A novel approach for reducing inaccessible and/or internalized bacterial populations is vacuum infiltration of sanitizing agents into apples.

MATERIALS AND METHODS

Fruit Samples Collected

Samples were collected at 12 apple orchards and 2 pear orchards throughout the U.S. (Table 1). A total of 8 different apple varieties (Cortland, Empire, Fuji, Golden Delicious, Granny Smith, Gala, McIntosh and Red Delicious) and two pear varieties (Bosc and D'Anjou) were collected. Twelve of the orchards visited were conventionally farmed, with the remaining 2 orchards (orchards 1 and 2; both apple orchards) organically farmed. One of the organic orchards visited in this survey was fertilized with composted manure, primarily chicken manure and other organic wastes. The other orchard was fertilized by an alfalfa cover crop.

A total of five categories of fruit, including tree fruit and dropped fruit, were collected. Fruit picked from the tree was designated either "calyx (i.e. blossom end) down" or "calyx up". By collecting fruit which had grown "calyx up" it could be determined if fruit oriented in this way had significantly greater microflora populations due to increased exposure of the calyx channel to potential sources of contamination from dust, contaminated water, etc., than fruit which had grown "calyx down". Pears do not commonly grow with the calyx oriented upwards, so this category was not included for this fruit. Fruit designated "damaged on tree", which had evidence of damage from bird pecks, hail or splitting during growth, etc., was collected. Dropped fruit including intact drops (designated "drops") and partially decayed drops (designated "drops with decay") were collected. Not all 5 categories of fruit were collected at each orchard, due to limited availability in some locations. A total of 63 fruit samples, comprising samples from all 5

categories described, were collected. Each sample consisted of 24 pieces of fruit, divided into 4 composites of 6, packed in individual polyethylene zip lock bags. Samples were packed in fruit boxes and transported to ERRC within 3 days. Fruit was stored at 4°C until analysis.

Sample Preparation

(a) Preparation of whole blend samples. Composite samples of six pieces of fruit were blended with an equal volume (w/v) of sterile 0.1% peptone water (PW), in duplicate. (b) Preparation of stem, core and calyx samples. Of the 63 fruit samples collected, duplicate sets of 45 of these were tested for internalization of microflora. Two composite samples of 6 apples or pears were used for this procedure. Stem, calyx and core pieces of each fruit were aseptically removed, and the remainder of the fruit was discarded. Samples consisting of 6 stem, core or calyx portions were individually blended in 4 parts PW (w/v). This procedure was performed in duplicate for each set of 6 stem, core and calyx portions.

Blended samples were filtered through a filter bag and 60 ml fruit filtrate (2 × 30 ml duplicate samples) from each blended sample was collected. Fruit filtrate samples (1 or 0.1 ml aliquots) were diluted as necessary in PW and plated (see below), with the remainder of the filtrate retained at 4°C.

Enumeration of Microbial Populations

Samples were enumerated for total mesophilic aerobic counts, total coliforms, generic *E. coli*, and yeasts and molds.

Total mesophilic aerobic counts were estimated by plating on Trypticase Soy Agar (TSA). TSA plates were incubated at 35°C for 24 h and manually counted.

Generic *E. coli* and total coliform counts were estimated by plating on *E. coli*/coliform count Petrifilm plates. Plates were incubated at 35°C and examined at 24 h and 48 h for the presence of coliforms (red colonies with gas) and generic *E. coli* (blue colonies with gas). Samples displaying these types of colonies were enriched to determine if they were positive for *E. coli* O157:H7. The enrichment procedure was as follows (P. M. Fratamico, personal communication): fruit filtrate samples (50 ml) were incubated in 200 ml of Trypticase Soy Broth (TSB) supplemented with Tween 20 (0.6% v/v). Samples were incubated and shaken at 37°C for 4 h. At this point, 0.02% (w/v) novobiocin was added to each sample, and was incubated for 20 h. The enriched samples were streaked (1 ml) on to Sorbitol MacConkey Agar, supplemented with 0.05-mg/l cefixime and 2.5 mg/l potassium tellurite (CT-SMAC) and incubated for 24 h at 35°C. Suspected O157:H7 colonies, which appeared colorless on CT-SMAC, were transferred to slants of TSA supplemented with 0.6 % yeast extract (TSAYE) and incubated overnight at 35°C. After incubation at 35C for 24 h, growth on TSAYE was tested for the production of indole by the spot test using filter paper wetted with Kovak's reagent. Indole positive isolates were tested for the O157 antigen with the RIM *E. coli* O157:H7 Latex Test.

Yeast and mold populations were estimated by plating 1 ml aliquots on Yeast and Mold count Petrifilm Plates. Plates were incubated at room temperature and counted manually at day three and five.

Environmental Samples Collected

Four soil samples (approx. 100 g) were collected from the perimeter and the interior of orchards 1-10 (Table 1). Duplicate samples (10 g) of soil were diluted in PW (90 ml), mixed using a stomacher for 1 min on medium speed, and filtered through a filter stomacher bag. The

resultant filtrate was diluted in PW as necessary and used for the enumeration of total mesophilic aerobic counts, total coliforms, and generic *E. coli* (see above)

Four irrigation water samples (approx. 10 ml) were collected from seven of the 14 orchards visited (Table 1). Water samples were diluted in PW as necessary and used for the enumeration of total mesophilic aerobic counts, total coliforms, and generic *E. coli*.

Fecal matter (approx. 100 g) was collected when observed. Duplicate samples (25 g) were added to TSB (225 ml) supplemented with Tween 20 (0.6% v/v) and the enrichment procedure for *E. coli* O157:H7 performed as described above.

Vacuum Infiltration of Sanitizing Agents

Apples were artificially inoculated with generic *E. coli* ATCC 25922, and held overnight at 4°C to dry. Composite samples (4 apples) were washed in 4 lit of a sanitizing agent at the appropriate temperature, under normal atmospheric pressure or one atmosphere of vacuum for 3 min. Whole blends of composite samples were then prepared as before, and used in determining residual populations of *E. coli* on apples.

RESULTS

Presence of *E. coli* in Fruit

Table 1 shows the microbial populations of fruit collected from U.S. orchards, autumn of 1999. The data reflect results obtained using the "whole blend" preparation method. No *E. coli* O157:H7 was detected in any fruit or environmental sample tested. Generic *E. coli* was detected in 6.3% (n = 4) of fruit samples tested (data not shown). Three of the four samples that were positive for generic *E. coli* were pears that had been damaged in some way. Two separate fruit samples from orchard 8, one "damaged on tree" and one "drop with decay" sample were positive for generic *E. coli* (populations = 0.40 and 0.70 $\log_{10}$ cfu/g respectively). One "drop with decay" sample from orchard 7 was positive for generic *E. coli* also (population = 0.85 $\log_{10}$ cfu/g). An intact tree picked apple from orchard 9 was also positive for generic *E. coli* (1.19 $\log_{10}$ cfu/g).

Effect of Orchard Management on Microbial Populations of Fruit

Orchard 13 had the highest overall total mesophilic aerobic count (5.29 $\log_{10}$ cfu/g) for whole fruit (Table 1). There was much evidence of deer in this location. However, total aerobic counts in orchard 13 were significantly greater than only orchard 11 ($P < 0.05$; Table 1).

Coliforms were detected in 74.6% of fruit samples tested. There was no significant difference in total coliform numbers between the orchards visited (range = 0.33-1.77 $\log_{10}$ cfu/g) (Table 1).

Yeast and mold counts were significantly higher ($P < 0.05$) (4.82 $\log_{10}$ cfu/g) in one of the organic orchards (orchard 2) than in orchards 3, 6, 9, 11, 12 and 14 (Table 1), probably due to the proliferation of these microflora in the absence of fungicides.

There was no significant difference in total aerobic counts (4.46 vs. 4.04 $\log_{10}$ cfu/g) or total coliform populations (0.80 vs. 1.14 $\log_{10}$ cfu/g) between organic and conventionally managed orchards, respectively.

Effect of Fruit Category Collected

Table 2 lists the microbial populations of fruit, based on the five categories collected, i.e calyx up, calyx down, damaged on tree, dropped, dropped with decay. Of these five categories of fruit, drops with decay had significantly higher total counts ($P < 0.05$), total coliforms ($P < 0.05$), and yeasts and molds ($P < 0.05$) than any other category collected (Table 2). Intact dropped fruit had significantly higher total counts ($P < 0.05$) and total coliforms ($P < 0.05$), than intact tree fruit (i.e. calyx up and calyx down samples). Damaged tree fruit had significantly higher total counts ($P < 0.05$) than intact tree fruit (Table 2). Only 59.3% of intact tree fruit were positive for total coliforms, compared to 83.9% of damaged on tree, dropped and dropped with decay samples (data not shown). There was no significant difference between microflora counts on intact tree fruit oriented calyx up or calyx down (Table 2).

Evidence of Internalization of Microflora

Table 3 lists shows the microbial populations of the, stem, core and calyx sections of the fruit. There were significantly higher total mesophilic aerobic counts ($P < 0.05$), total coliform counts ($P< 0.05$), and yeast and mold counts ($P< 0.05$) in the stem and the calyx sections, than in the core (Table 3). Greater numbers of total mesophilic aerobic flora and total coliforms were internalized within dropped and damaged fruit ($P < 0.05$) than within intact tree fruit (Table 3). There was no significant difference between the number of microorganisms internalized in fruit oriented calyx up or calyx down on the tree (Table 3). Coliforms were detected in 40% of core samples, in contrast to 64.4% of calyx samples and 73.3% of stem samples tested. Generic *E coli* was internalized within the core of one dropped decayed sample (data not shown). This was probably an artifact of the actual piece of fruit. It was observed that dropped decayed fruit was often very decomposed, making exact distinctions between stem, core and calyx portions very difficult.

Microbial Populations in the Orchard Environment

Table 4 shows the microflora populations of the soil and irrigation water samples collected. Generic *E. coli* was detected in soil samples from orchards 3, 6, 7 and 9, i.e. in 4 of 10 orchards where soil samples were collected (Table 4). Total mesophilic aerobic counts in soil samples collected from the orchards visited ranged from 4.86 (orchard 8) to 6.67 $\log_{10}$ cfu/g (orchard 7). The high counts observed in orchard 7 may be a result of using cow manure for fertilization in this orchard. Orchard 9 had significantly ($P < 0.05$) higher total coliforms than the other orchards tested (Table 4). This high coliforms count may be the result of close proximity of this orchard to a pasture. Both orchards 7 and 9 were associated with the presence of generic *E. coli* on fruit (data not shown).

Irrigation water samples were collected from 7 of the 14 orchards visited (Table 4). Water samples from orchards 9, 11 and 13 were positive for generic *E. coli*, with populations ranging from < 0.18 (the lower limit of detection) to 0.40-log10 cfu/ml.

Fecal matter was collected in 6 of the 14 orchards visited (Table 1). *E. coli* O157:H7 was not detected in any of these samples.

Vacuum Infiltration of Sanitizing Agents

Table 5 shows the efficacy of vacuum infiltration of sanitizing agents into apples artificially inoculated with non-pathogenic *E. coli*. Washing treatments using 5% hydrogen peroxide was superior to that of 200-ppm chlorine solution (Table 5). Vacuum treatment with 5% hydrogen

peroxide resulted in an increase in *E. coli* reduction (1.4-fold) as compared to no vacuum treatment (Table 5).

SUMMARY

- No *E. coli* O157:H7 was isolated.
- Orchards with evidence of deer, fecal contamination, and proximity to pastures were associated with the presence of generic *E. coli* and had higher total aerobic counts and higher total coliforms than other orchards.
- Dropped and damaged fruit had higher total aerobic and higher total coliforms than intact fruit.
- *E. coli* is capable of surviving on tree fruit.
- Microbial populations in the stem and calyx areas were similar.
- The level of microflora detected within the core varied significantly depending on the area where the sample was collected.
- Higher levels of microflora were internalized in dropped and damaged fruit.
- 5% hydrogen peroxide washing treatment was superior to 200-ppm chlorine washing treatment in reducing *E.* coli populations on artificially inoculated apples.
- Vacuum infiltration of hydrogen peroxide into artificially inoculated apples seemed to increase the efficacy of this sanitizing agent in reducing *E. coli* populations.

CONCLUSIONS

- Orchards should be located away from pasture lands, and fences should be erected to keep out wild and domestic animals.
- Water used in artificial irrigation should be certified potable, if not, should be monitored frequently for indicator microorganisms.
- Dropped and/or damaged fruit should not be included in the production of unpasteurized apple cider.

Table 1. Microbial Populations (log_{10} cfu/g) of Fruit Collected from U. S. Orchards, Autumn 1999

Location	Name	Variety	Total aerobic counts	Total coliforms	Yeasts and molds
Pacific NW	1 [a, b]	Red Delicious	3.94 *(1.27)* ABC	0.71 *(1.03)* A	4.66 *(0.56)* ABC
Pacific NW	2 [a]	Gala	4.93 *(1.04)* A	0.86 *(0.83)* A	4.82 *(0.56)* A
Pacific NW	3	Golden Delicious	3.97 *(1.01)* ABC	0.50 *(0.74)* A	4.02 *(0.66)* CDE
Pacific NW	4 [b]	Red Delicious	4.14 *(0.92)* ABC	1.58 *(1.06)* A	4.26 *(0.50)* ABCDE
Pacific NW	5 [b, c]	Granny Smith	5.03 *(1.43)* A	1.48 *(1.30)* A	4.59 *(0.65)* ABCD
Pacific NW	6	Fuji	4.88 *(1.38)* A	1.02 *(1.26)* A	4.13 *(0.46)* BCDE
Pacific NW	7 [b]	D'Anjou (pears)	3.81 *(2.72)* ABC	1.53 *(1.43)* A	4.27 *(0.77)* ABCDE
Pacific NW	8 [c]	Bosc (pears)	2.93 *(1.79)* BC	0.63 *(0.82)* A	4.24 *(0.44)* ABCDE
Midwest	9 [b, d]	Golden Delicious and Granny Smith	3.85 *(1.11)* ABC	1.12 *(1.00)* A	ND
Northeast	10 [b, c]	Mackintosh	4.02 *(1.03)* ABC	1.67 *(1.79)* A	3.93 *(0.66)* DE
Northeast	11 [b, c]	Golden Delicious	2.73 *(0.88)* C	0.33 *(0.62)* A	3.92 *(0.15)* DE
Northeast	12	Empire and Red Delicious	4.40 *(0.67)* AB	1.15 *(1.22)* A	4.02 *(0.10)* CDE
Northeast	13 [b, c]	Cortland	5.29 *(0.36)* A	1.77 *(1.39)* A	4.76 *(0.90)* AB
Northeast	14 [c]	Cortland and Empire	3.68 *(0.76)* ABC	1.05 *(1.10)* A	3.91 *(0.11)* E

Data obtained using 'whole blend' preparation method. Means in each column followed by different letters are significantly different ($P<0.05$). Standard deviations are shown in parenthesis. [a] Organically managed orchards. [b] Irrigation water collected. [c] Fecal matter collected. [d] *E. coli* located on calyx down Golden Delicious apple. ND = not done.

Table 2. Effect of Fruit Category Collected on Microbial Populations (log_{10} cfu/g)

Category	Total counts	Total coliforms	Yeasts and molds
Calyx down	3.12 *(1.08)* A	0.46 *(0.72)* A	4.02 *(0.35)* A
Calyx up	3.67 *(1.02)* A	0.67 *(0.92)* A	4.10 *(0.53)* A
Damaged on tree	4.42 *(1.13)* B	0.88 *(1.17)* A	4.29 *(0.55)* A
Drops	4.47 *(1.21)* B	1.74 *(1.04)* B	4.17 *(0.63)* A
Drops with decay	5.94 *(0.83)* C	2.08 *(1.49)* B	5.11 *(0.44)* B

Data obtained using 'whole blend' preparation method. Means in each column followed by different letters are significantly different ($P < 0.05$). Standard deviations are shown in parenthesis.

Table 3. Microbial Populations (log_{10} cfu/g) of the Calyx, Core and Stem Sections of the Five Fruit Categories Sampled

Category	Fruit section	Total aerobic counts	Total coliforms	Yeasts and Molds
Calyx down	Calyx	3.34*(1.30)*A	0.60*(1.06)*A	5.04*(0.55)*A
	Core	1.37*(1.15)*B	0.10*(0.45)*A	2.14*(1.00)*B
	Stem	3.32*(1.46)*A	0.32*(0.62)*A	4.51*(0.61)*A
Calyx up	Calyx	3.94*(0.84)*A	0.48*(0.92)*A	5.06*(0.58)*A
	Core	1.36*(1.03)*B	0.09*(0.38)*A	2.53*(1.16)*B
	Stem	3.11*(1.01)*A	0.87*(1.09)*A	4.48*(0.69)*A
Damaged on tree	Calyx	4.12*(1.07)*A	1.02*(1.18)*A	4.97*(0.46)*A
	Core	2.32*(1.66)*B	0.64*(1.11)*A	2.38*(1.25)*B
	Stem	4.18*(1.54)*A	1.44*(1.24)*A	4.61*(0.54)*A
Drops	Calyx	4.58*(1.15)*A	1.69*(1.23)* A	5.20*(0.36)*A
	Core	2.39*(1.16)*B	0.40*(0.84)*B	2.61*(1.06)*B
	Stem	4.66*(0.94)*A	1.53*(1.11)*A	4.77*(0.57)*A
Drops with decay	Calyx	5.33*(0.80)*A	1.60*(1.14)*A	5.26*(0.70)*A
	Core	4.33*(1.14)* A	1.47*(1.07)*A	3.96*(0.91)*B
	Stem	5.23*(1.05)*A	1.50*(0.99)*A	5.36*(0.56)*A

Means in each column, within each category, followed by different letters are significantly different ($P<0.05$). Standard deviations are shown in parenthesis.

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	Core	1.37*(1.15)*B	0.10*(0.45)*A	2.14*(1.00)*B
	Stem	3.32*(1.46)*A	0.32*(0.62)*A	4.51*(0.61)*A
Calyx up	Calyx	3.94*(0.84)*A	0.48*(0.92)*A	5.06*(0.58)*A
	Core	1.36*(1.03)*B	0.09*(0.38)*A	2.53*(1.16)*B
	Stem	3.11*(1.01)*A	0.87*(1.09)*A	4.48*(0.69)*A
Damaged on tree	Calyx	4.12*(1.07)*A	1.02*(1.18)*A	4.97*(0.46)*A
	Core	2.32*(1.66)*B	0.64*(1.11)*A	2.38*(1.25)*B
	Stem	4.18*(1.54)*A	1.44*(1.24)*A	4.61*(0.54)*A
Drops	Calyx	4.58*(1.15)*A	1.69*(1.23)*A	5.20*(0.36)*A
	Core	2.39*(1.16)*B	0.40*(0.84)*B	2.61*(1.06)*B
	Stem	4.66*(0.94)*A	1.53*(1.11)*A	4.77*(0.57)*A
Drops with decay	Calyx	5.33*(0.80)*A	1.60*(1.14)*A	5.26*(0.70)*A
	Core	4.33*(1.14)*A	1.47*(1.07)*A	3.96*(0.91)*B
	Stem	5.23*(1.05)*A	1.50*(0.99)*A	5.36*(0.56)*A

Means in each column, within each category, followed by different letters are significantly different ($P<0.05$). Standard deviations are shown in parenthesis.

Table 4. Microbial Populations (log_{10} cfu/g) of Soil and Irrigation Water Collected

Location	Name	Soil			Irrigation water		
		Total aerobic counts	Total coliforms	*E. coli*	Total aerobic counts	Total coliforms	*E. coli*
Pacific NW	1 [a]	5.79BC	1.81BCDE	nd A	0.50	Nd	nd
Pacific NW	2 [a]	5.83BC	1.85BCDE	nd A	-	-	-
Pacific NW	3	5.69BC	1.01DEFG	0.13A	-	-	-
Pacific NW	4	6.07B	nd G	nd A	1.75	Nd	nd
Pacific NW	5	5.55C	0.42GF	nd A	0.16	Nd	nd
Pacific NW	6	5.63BC	2.27BCD	0.65A	-	-	-
Pacific NW	7	6.67A	2.79B	0.21A	1.75	0.48	Nd
Pacific NW	8	4.86D	1.47CDEF	nd A	-	-	-
Midwest	9	5.68BC	4.31A	0.32A	3.34	0.74	0.18 [b]
Northeast	10	5.61C	0.67EFG	nd A	-	-	-
Northeast	11	-	-	-	2.96	0.45	0.18
Northeast	12 [c] to 14	-	-	-	4.20	4.20	0.75

Means in each column followed by different letters are significantly different ($P < 0.05$). [a] Organically managed orchards. [b] 0.18 cfu was the lower limit of detection for this technique. [c] Sample taken from creek which supplies irrigation water to all these orchards. Nd = not detected. – = analysis was not performed.

Table 5. Vacuum Infiltration of Sanitizing Agents into Golden Delicious Apples Inoculated with *E. coli* ATCC 25922

Treatment	Population Reduction [a] (log_{10} cfu/g)
5% H_2O_2 wash @ 60C	2.26 (0.41)
5% H_2O_2 vacuum @ 45C	3.12 (0.70)
200 ppm Cl_2 wash @ 50C	1.48 (0.25)
200 ppm Cl_2 vacuum @ 45C	1.53 (0.12)

[a] Based on inoculated control *E. coli* population of 5.1 log_{10} cfu/g. Standard deviations are shown in parenthesis.